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## (57) Abstract

A method of modulating the ripening and/or senescence characteristics in plants of the genus *Musa* comprises transforming plants with one or more sequences obtainable from the deposited cDNA library having the accession number 40184, regenerating said plants and selecting from the population of transformants those plants having modulated and/or tissue senescence characteristics.

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GENETIC CONTROL OF FRUIT RIPENING

This invention relates generally to the modification of a plant phenotype by the regulation of plant gene expression. More specifically it relates to the modulation of the ripening and/or tissue senescence characteristics and plants derived therefrom. One suitable application of the present invention is the modulation of ripening and/or senescence processes in plants of the genus *Musa* (referred to herein as banana).

Two principal methods for the control of expression are known, viz.: overexpression and underexpression. Overexpression may be achieved by insertion of one or more than one extra copies of the selected gene. It is, however, not unknown for plants or their progeny, originally transformed with one or more than one extra copy of a nucleotide sequence, to exhibit the effects of underexpression as well as overexpression.

For underexpression, often referred to as "gene silencing", there are two principle methods which are commonly referred to in the art as "antisense downregulation" and "sense downregulation (also referred to as "cosuppression"). Both of these methods lead to an inhibition of expression of the target gene. Other lesser used methods involve modification of the genetic control elements, the promoter and control sequences, to achieve greater or lesser expression of an inserted gene.

There is no reason to doubt the operability of these methods: they are well- established, used routinely in laboratories around the world and products in which they have been used are on the market.

Gene control by any of these methods requires the insertion of a selected gene or genes into plant material which can be regenerated into plants. This transformation process can be performed via a number of methods, for example: the *Agrobacterium*-mediated transformation method.

In the microparticle bombardment method, microparticles of dense material, usually gold or tungsten, are fired at high velocity at the target cells where they penetrate the cells, opening an aperture in the cell wall through which DNA may enter. The DNA may be coated on to the microparticles or may be added to the culture medium.

In microinjection, the DNA is inserted by injection into individual cells via an ultrafine hollow needle.

Another method, viz. fibre-mediated transformation, applicable to both monocots and dicots, involves creating a suspension of the target cells in a liquid, adding microscopic needle-like material, such as silicon carbide or silicon nitride "whiskers", and agitating so that the cells and whiskers collide and DNA present in the liquid enters the cell.

5 In summary, then, the requirements for both sense and antisense technology are known and the methods by which the required sequences may be introduced are known. What remains, then is to identify genes whose regulation will be expected to have a desired effect, isolate them or isolate a fragment of sufficiently effective length, construct a chimeric gene in which the effective fragment is inserted between promoter and termination signals, and insert the  
10 construct into cells of the target plant species by transformation. Whole plants may then be regenerated from the transformed cells.

Bananas are a globally important fruit crop. They are not only a popular dessert fruit, but represent a vital carbohydrate staple in the tropics with as many as 100 million people  
15 subsisting on bananas and plantains as their main energy source. The cultivated dessert banana is commonly triploid, parthenocarpic and belongs to the *Musa* AAA genome group, eg. Cavendish subtypes. Bananas are climacteric fruits and ripening is regulated by ethylene produced by the fruit and involves numerous biochemical changes including the conversion of starch to sugars, cell wall disassembly, synthesis of volatile compounds, changes in  
20 phenolic constituents and degradation of chlorophyll in the peel. The conversion of starch to sugars is particularly striking, where starch accounts for 20-25% of the fresh weight of the unripe fruit and depending on the genetic background, can be converted almost entirely to sugars.

The triploid nature of the cultivated dessert banana crop has hampered conventional methods  
25 of breeding for improved characteristics. As a result of this an enormous pool of genetic resources for enhancing postharvest characteristics of the fruit has remained untapped.

According to the present invention there is provided a method of modulating the ripening or tissue senescence process in plants of the genus *Musa* comprising inserting into plant  
30 material at least one polynucleotide sequence selected from the sequences depicted as [SEQ ID-Nos. 1 -57] <sup>SEQ ID NOS: 1-51</sup> regenerating said plant material and selecting from the transformed

regenerants, plants with modulated ripening or tissue senescence characteristics. The said polynucleotide may be obtained from the cDNA library having the NCIMB Accession Number 40814.

Further according to the present invention is a method of modulating the ripening or tissue senescence process in plants of the genus *Musa* comprising inserting into plant material at least one polynucleotide sequence or a fragment thereof, obtainable by hybridisation, from the cDNA library having the NCIMB Accession Number 40814, by the use of at least one of the sequences depicted as SEQ ID Nos 1-57 <sup>✓</sup> SEQ ID NOS: 1-57 as oligonucleotide probes, said hybridisation being conducted at a temperature from 60°C to 65°C in 0.3 strength citrate buffered saline containing 0.1% SDS followed by rinsing at the same temperature with 0.3 strength citrate buffered saline containing 0.1% SDS, regenerating said plant material and selecting from the transformed regenerants, plants with modulated ripening or tissue senescence characteristics. The invention further provides a method as described above wherein the said polynucleotide is capable of modulating the production of pectate lyase and more specifically the polynucleotide comprises at least one of the sequences depicted in the sequence listings as SEQ-ID-Nos. 13-18 <sup>✓</sup> SEQ ID NOS: 13-18

A preferred method for inserting the said polynucleotides into plant material according to the method of the present invention, may be selected from the group comprising the *Agrobacterium*, microparticle bombardment, fibre mediated or direct insertion methods.

The invention further provides plants, their progeny and seed and material obtained from said plants, produced according to the method of the present invention. It is preferred that the said plants, their progeny and seed and material obtained from said plants are derived from plants of the genus *Musa*.

The present invention also provides a vector functional in plants comprising a promoter region which is operable in plant cells, at least one of the polynucleotide sequences described above and a transcription termination sequence. The promoter may be constitutive, developmentally regulated or switchable. In addition to this the promoter may also be tissue specific or organ specific.

Further provided is a banana produced via the preceding method, having altered fruit characteristics when compared with a banana which is not transformed with at least one of the polynucleotide sequences described above.

The present invention also provides an improved method of controlling plant pathogens comprising the application of an anti-pathogenic agent to plants, characterised in that plants to which the said agent is applied, are plants according to the present invention.

The gene sequences of the present invention may be synthesised *ab initio*, using the sequence data in the sequence listing provided herewith, or isolated from a library using the standard techniques known within the art. The sequences depicted in the sequence listing or parts thereof may also be used to create oligonucleotide probes for the purposes of isolating from the library those polynucleotides which are capable of producing the desired proteins. To assist the isolation of these polynucleotides we have deposited with the National Collection of Industrial & Marine Bacteria, St. Machar Drive, Aberdeen, UK, a cDNA library of the banana peel ripening related genes. The library was deposited on 9th July 1996 and has the NCIMB Accession Number 40814.

Thus, this invention is based on the identification of genes which encode proteins involved in banana ripening-related processes, specifically within banana pulp. DNA sequences according to the sequence listing or those sequences obtainable from the deposited library, may be used in the process of modifying the plant ripening characteristics of plants and/or fruit.

By virtue of this invention banana plants can be generated which, amongst other phenotypic modifications, may have one or more of the following fruit characteristics: improved resistance to damage during harvest, packaging and transportation due to slowing of the ripening and over-ripening processes; longer shelf life and better storage characteristics due to reduced activity of degradative pathways (e.g. cell wall hydrolysis); improved processing characteristics due to changed activity of proteins/enzymes contributing to factors such as: viscosity, solids, pH, elasticity; improved flavour and aroma at the point of sale due to modification of the sugar/acid balance and other flavour and aroma components responsible for characteristics of the ripe fruit; modified colour due to changes in activity of enzymes involved in the pathways of pigment biosynthesis (e.g. lycopene,  $\beta$ -carotene, chalcones and anthocyanins); increased resistance to post-harvest pathogens such as fungi.

The activity of the ripening-related proteins may be either increased or reduced depending on the characteristics desired for the modified plant part (fruit, leaf, flower, etc). The levels of protein may be increased; for example, by incorporation of additional genes. The additional

genes may be designed to give either the same or different spatial and temporal patterns of expression in the fruit. "Antisense" or "partial sense" or other techniques may be used to reduce the expression of ripening-related protein.

5 The activity of each ripening-related protein or enzyme may be modified either individually or in combination with modification of the activity of one or more other ripening-related proteins/enzymes. In addition, the activities of the ripening-related proteins/enzymes may be modified in combination with modification of the activity of other enzymes involved in fruit ripening or related processes.

10 DNA constructs according to the invention for gene silencing, may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence, it may be as long as the relevant mRNA produced by the cell but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. When using genomic DNA as the source of a base sequence for transcription it is possible to use either intron or exon regions or a combination of both.

15 As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used. The isolation of suitable ripening-related sequences is described above; it is convenient to use DNA sequences derived from the ripening-related clones deposited at NCIMB in Aberdeen. Sequences coding for the whole, or substantially the whole, of the appropriate ripening-related protein may thus be obtained. Suitable lengths of this DNA sequence may be cut out for use by means of restriction enzymes. The preparation of such constructs is described in more detail below.

20 Constructs suitable for expression of the appropriate ripening-related sequence in banana cells, may be produced using a cDNA sequence selected from the deposited library having the NCIMB Accession Number 40814 or the gene sequence as found in the chromosome of the banana plant. Recombinant DNA constructs may be made using standard techniques. In this specification modulation means an increase or decrease of the desired effect. More specifically "modulation of the ripening or tissue senescence process in plants of the genus *Musa*" means an increase or decrease in production of a ripening related protein

25

30 resulting from the method as described above. For example, where an increased ripening related protein is desired, plants may be transformed according to the method as described

above and those plants exhibiting the desired effect may be selected from the population of transformants. Furthermore, it may be desirable to provide a plant with modulated ripening or tissue senescence characteristics by increasing the production of one protein and decreasing the production of another protein in the same plant. For example, a banana fruit, modified using the present method, and having decreased levels of the enzyme pectate lyase would be beneficial because pulp softening would require a substantially longer time when compared with a control. In addition to this and by increasing the levels of another ripening related protein such as an antifungal protein in the same fruit using the present method, would complement the extended life of the banana pulp with increased resistance to disease.

Increase in production of a ripening related protein includes inserting into plant material one or more copies of any of the polynucleotides described above, wherein the said polynucleotides must be capable of producing a protein and thereby increasing protein levels when compared with a control plant.

Decrease in production of a ripening related protein includes inserting into plant material one or more copies of any of the polynucleotides described above, wherein the said polynucleotides must be capable of producing an mRNA which is capable of interfering with endogenous mRNA to such a degree that the levels of translated protein are reduced when compared with a control plant.

Ripening process of plants means the process of maturing or developing .

Senescence means the progressive deterioration in function of cells, tissues, organs etc., related to the period of time since that function commenced.

Control plant means a comparable plant used for the purposes of determining modulation of the ripening or tissue senescence process effect in plants. Specifically, in plant transformations the control plant is usually of the same species and variety as the material used before the transformation process and is grown in the same conditions, (usually with the transformant selection step modified in some way on the part of the control plant), as the transformed plants. More specifically the control plant may comprise an untransformed control plant or a transformed control plant providing it has not already been transformed with the same polynucleotide sequence as the plant material to be transformed.

"Plant material" includes plant cells and any other type of plant regenerable material.

The following examples further illustrate the invention but are not to be construed to limit the scope thereof:

TABLE 1. Is a list of clones isolated from banana pulp and the corresponding sequence identity number as provided in the sequence listing herein. The table also illustrates the approximate clone size, the percentage identity and, where relevant, nucleotide similarity with published sequences, based on the results obtained from comparisons with the EMBL sequence database. The table also provides, where relevant, the gene identity of those published sequences and their database accession numbers.

FIGURE 1. Plant transformation vector pUN, containing the UBI polyubiquitin promoter.

FIGURE 2. Plant transformation vector pSHYN, containing hygromycin resistance gene for selection of transformed plants.

FIGURE 3. Plant transformation vector pAN, containing the banana ACC oxidase promoter.

#### EXAMPLE 1

##### Construction of a cDNA library of ripening genes

###### 1.1 Isolation of messenger RNA

Total RNA was isolated from ripening (24 hours after ethylene treatment) banana pulp (*Musa acuminata* cv. Grand Nain) as described by Chang et al, Plant Molecular Biology Reporter, Vol. 11(2) 113-116 (1993). Messenger RNA was isolated from total RNA by Oligo(dT)-cellulose chromatography according to Bantle et al., Analytical Biochemistry 72, 413-427 (1976).

###### 1.2 Synthesis of cDNA and Cloning into Vector

The first and second strands of the cDNAs were synthesised from the messenger RNAs using a commercial cDNA synthesis kit (Catalog No. 200450, ZAP Express™ Gold Cloning kit, Stratagene Ltd, Cambridge, Cambs, UK). Double stranded cDNAs were cloned into the ZAP Express™ vector, packaged, mixed with plating bacteria to determine titre and for library screening, following instructions of the suppliers protocol.



### 1.3 Screening of the cDNA library from banana pulp.

The unamplified cDNA library from ripening banana pulp was differentially screened using cDNA from unripe and ripening banana peel tissue. A proportion of the library was plated individually at low density and duplicate plaque lifts made onto Hybond N nylon filters (Amersham) according to the manufacturer's instructions. One filter was hybridised to dCTP radiolabelled cDNA from green fruit and the duplicate filter hybridised to dCTP radiolabelled cDNA from ripening fruit. Hybridization's were at high stringency. Plaques hybridising preferentially with ripening or green radiolabelled cDNA were picked and re-plated for a second round of selection by differential screening. These clones were numbered as ripening up- or down-regulated peel clones. The clones were in-vivo excised from the ZAP express<sup>TM</sup> vector into the pBK-CMV phagemid vector using the ExAssist<sup>TM</sup> interference-resistant helper phage, following instructions from manufacturers protocol.

### 1.4 Characterisation of the ripening pulp cDNA library and the ripening-related clones.

The ripening cDNA library from pulp tissue were prepared with an efficiency of  $3.2 \times 10^5$  plaque-forming units per microgram of cDNA. The sizes of the inserts in the peel library was 0.4 - 6.7 Kb with a mean size insert of 1.47 Kb.

From the 250 plaques used in the first screen, 73 putative ripening-related clones were obtained. These 73 clones were partially sequenced using the ABI PRISMTM Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq<sup>®</sup> DNA polymerase (Applied Biosystems, Warrington, Cheshire, UK) with forward primers specific for the pBK-CMV vector. From these, the following ripening-related clones were selected. Comparisons of these sequences in the EMBL database using GCG ('Wisconsin') software has identified homologies for the clones listed in TABLE 1 below.

## EXAMPLE 2

Construction of truncated sense RNA vectors with the maize polyubiquitin promoter.

A vector is constructed using the sequences corresponding to a fragment of the inserts of one of the sequences 1 to 57. This fragment is synthesised by polymerase chain reaction using synthetic primers incorporating BamHI restriction sites suitable for cloning between a

maize UBI polyubiquitin promoter (Christensen et al, 1992, Plant Molecular Biology, 18:675-689) and a nopaline synthase 3' end termination sequences in the vector pUN (Fig. 1.).

The truncated sense expression cassette is excised by digestion with *AscI*, the ends of the fragment are made flush with T4 polymerase and it is cloned into the vector pSHYN (Fig. 2.) which has been cut with *KpnI* and the ends made flush with Klenow polymerase. pSHYN contains hygromycin resistance gene for selection of transformed plants.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

### EXAMPLE 3

Construction of truncated sense RNA vectors with a fruit enhanced promoter.

The 1386bp *HindIII* fragment containing the banana ACC oxidase promoter (UK. Application No. 9607700.3) is cloned the *HindIII* site in pMSC2 (Fig. 3.) to give the vector pAN.

A vector is constructed using the sequences corresponding to a fragment of the inserts of one of the sequences 1 to 57. This fragment is synthesised by polymerase chain reaction using synthetic primers incorporating *BamHI* restriction sites suitable for cloning between a maize UBI polyubiquitin promoter (Christensen et al, 1992, Plant Molecular Biology, 18:675-689) and a nopaline synthase 3' end termination sequences in the vector pAN.

The truncated sense expression cassette is excised by digestion with *AscI*, the ends of the fragment are made flush with T4 polymerase and it is cloned into the vector pSHYN (Fig. 2.) which has been cut with *KpnI* and the ends made flush with Klenow polymerase. pSHYN contains hygromycin resistance gene for selection of transformed plants.

After synthesis of the vector, the structure and orientation of the sequences are confirmed by DNA sequence analysis.

### EXAMPLE 4

Construction of an over-expression vector with the maize polyubiquitin promoter.

The complete sequence of a ripening -related cDNA containing a full open-reading frame is inserted into the vectors described in EXAMPLE 2.

#### EXAMPLE 5

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Construction of an over-expression vector with a fruit enhanced promoter.

The complete sequence of a ripening -related cDNA containing a full open-reading frame is inserted into the vectors described in EXAMPLE 3.

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#### EXAMPLE 6

Generation of transformed Musa plants

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Transformed Musa plants containing the vectors are produced by the method described in Sagi et al. (1995) Biotechnology. Vol. 13 pp 481-485. Regenerated transformed plants are identified by their ability to grow on hygromycin and grown to maturity. Ripening fruit are analysed for a modulation in their ripening related or senescence characteristics.

20

Other suitable transformation methods for banana are described in Sagi et al. (1994) Plant Cell Reports. Vol. 13. pp 262-266. and May et al. (1995) Biotechnology. Vol. 13 pp 486-492.

TABLE 1

Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ-ID-NO-1	Pulp Upregulated	U-U9	1	Isoflavone Reductase	61.6	714	Potato ( <i>Solanum tuberosum</i> ), X92075
SEQ-ID-NO-2	Pulp Upregulated	U-U17	1	Isoflavone Reductase	62.9	770	Potato ( <i>Solanum tuberosum</i> ), X92075
SEQ-ID-NO-3	Pulp Upregulated	U-U66	1.1	Isoflavonoid Reductase	60.0	722	<i>Arabidopsis thaliana</i> , Z49777
SEQ-ID-NO-4	Pulp Upregulated	U-U104	1	Isoflavonoid Reductase	60.6	696	<i>Arabidopsis thaliana</i> , Z49777
SEQ-ID-NO-5	Pulp Upregulated	U-U13	1.1	Beta-1,3-Glucanase	58.5	585	Soybean ( <i>Glycine max</i> ), A26451
SEQ-ID-NO-6	Pulp Upregulated	U-U136	1.3	Beta-1,3-Glucanase	59.8	800	Barley ( <i>Hordeum vulgare</i> ), M91814
SEQ-ID-NO-7	Pulp Upregulated	U-U21	1.8	Transcriptional Activator	54.3	311	<i>Zea mays</i> , L13454

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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ-ID-NO-8	Pulp Upregulated	U-U31	0.55	S-adenosylhomocysteine Hydrolase	67.0	467	Orchid ( <i>Phalaenopsis</i> sp.), X79905
SEQ-ID-NO-9	Pulp Upregulated	U-U131	1.8	S-adenosyl L-homocysteine Hydrolase	79.8	699	Wheat ( <i>Triticum aestivum</i> ), L11872
SEQ-ID-NO-10	Pulp Upregulated	U-U32	0.8	Beta-amylase	54.7	854	<i>Zea mays</i> , Z25871
SEQ-ID-NO-11	Pulp Upregulated	U-U55	0.8	O-methyl transferases	60.1	223	<i>Zea mays</i> , L14063
SEQ-ID-NO-12	Pulp Upregulated	U-U72	0.8	O-methyl transferase	60.6	226	<i>Zea mays</i> , L14063
SEQ-ID-NO-13	Pulp Upregulated	U-U68	1.8	Pectate Lyase	57.9	394	<i>Lilium longiflorum</i> , Z17328
SEQ-ID-NO-14	Pulp Upregulated	U-U69	1.7	Pectate Lyase	66.1	516	<i>Zea mays</i> , L20140
SEQ-ID-NO-15	Pulp Upregulated	U-U84	1.1	Pectate Lyase	65.8	736	<i>Lycopersicon esculentum</i> , X55193
SEQ-ID-NO-16	Pulp Upregulated	U-U89	1.6	Pectate Lyase	54.8	354	<i>Nicotiana tabacum</i> , X67158

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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ -ID-NO-17	Pulp Upregulated	U-U115	1.5	Pectate Lyase	67.3	508	<i>Zea mays</i> , L20140
SEQ -ID-NO-18	Pulp Upregulated	U-U117	1.7	Pectate Lyase	55.0	333	<i>Lilium longiflorum</i> , Z17328
SEQ -ID-NO-19	Pulp Upregulated	U-U80	0.6	Vicilin-like storage protein	53.7	387	<i>Arabidopsis thaliana</i> , T13642
SEQ -ID-NO-20	Pulp Upregulated	U-U90	1.8	Glutamate Decarboxylase	72.5	735	<i>Petunia hybrida</i> , L16797
SEQ -ID-NO-21	Pulp Upregulated	U-U92	1.8	Glutamate Decarboxylase	71.8	740	<i>Arabidopsis thaliana</i> , U10034
SEQ -ID-NO-22	Pulp Upregulated	U-U91	1.1	Aconitase	76.4	766	Pumpkin ( <i>Cucurbita sp.</i> ), D29629
SEQ -ID-NO-23	Pulp Upregulated	U-U96	0.9	Express Sequence Tag.	69.0	452	<i>Arabidopsis thaliana</i> , H36910
SEQ -ID-NO-24	Pulp Upregulated	U-U103	2	Cell Wall Invertase	66.0	567	<i>Zea mays</i> , U17695
SEQ -ID-NO-25	Pulp Upregulated	U-U93	3.5	Heat Shock Protein cognate	76.4	711	<i>Lycopersicon esculentum</i> , X54030
SEQ -ID-NO-26	Pulp Upregulated	U-U125	1.8	Heat Shock Protein	71.0	662	<i>Spinacia oleracea</i> , L26243
SEQ -ID-NO-27	Pulp Upregulated	U-U105	0.9	Hexa ubiquitin Protein	64.1	237	Sunflower ( <i>Helianthus annuus</i> ), X57004

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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ-ID-NO-28	Pulp Upregulated	U-U116	0.8	Polyubiquitin	81.0	625	Rice ( <i>Oryza sativa</i> ), X76064
SEQ-ID-NO-29	Pulp Upregulated	U-U120	0.9	Extensin	56.9	576	Almond ( <i>Prunus amygdalus</i> ), X65718
SEQ-ID-NO-30	Pulp Upregulated	U-U126	1.2	Basic Chitinase	63.2	506	Cowpea ( <i>Vigna unguiculata</i> ), X88801
SEQ-ID-NO-31	Pulp Upregulated	U-U129	1.3	Beta-glucosidase	60.9	517	White clover ( <i>Trifolium repens</i> ), X56733
SEQ-ID-NO-32	Pulp Upregulated	U-U130	1.3	Chlorophyll a/b binding protein	52.0	415	Rice ( <i>Oryza sativa</i> ), D00641
SEQ-ID-NO-33	Pulp Upregulated	U-U43	2.5	Fruit sp pp involved in maturation	53.8	396	Tomato ( <i>Lycopersicon esculentum</i> ), X13743
SEQ-ID-NO-34	Pulp Upregulated	U-U70	0.6	Root specific protein	63.6	420	Rice ( <i>Oryza sativa</i> ), L27208
SEQ-ID-NO-35	Pulp Upregulated	U-U16	0.6	-	-	-	No published sequence similarity
SEQ-ID-NO-36	Pulp Upregulated	U-U30	0.8	-	-	-	-
SEQ-ID-NO-37	Pulp Upregulated	U-U40	1.0	-	-	-	-
SEQ-ID-NO-38	Pulp Upregulated	U-U108	1.1	-	-	-	-

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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ-ID-NO-39	Pulp Upregulated	U-U128	2.2	-	-	-	-
SEQ-ID-NO-40	Pulp Down regulated	U-D4	1.3	Granule Bond Starch Synthase	62.3	621	Cassava ( <i>Manihot esculenta</i> ), X74160
SEQ-ID-NO-41	Pulp Down regulated	U-D10	1.3	Starch Synthase	68.8	506	Pea ( <i>Pisum sativum</i> ), X88789
SEQ-ID-NO-42	Pulp Down regulated	U-D13	1.3	Starch Synthase	67.4	454	Pea ( <i>Pisum sativum</i> ), X88789
SEQ-ID-NO-43	Pulp Down regulated	U-D66	1.3	Granule Bond Starch Synthase	64.1	669	Cassava ( <i>Manihot esculenta</i> ), X74160
SEQ-ID-NO-44	Pulp Down regulated	U-D111	1.3	Starch Synthase	66.4	655	Cassava ( <i>Manihot esculenta</i> ), X74160
SEQ-ID-NO-45	Pulp Down regulated	U-D112	2.3	Granule Bond Starch Synthase	68.4	196	Potato ( <i>Solanum tuberosum</i> ), X58453



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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ -ID-NO-46	Pulp Down regulated	U-D39	1.1	Antifungal Protein	69.1	517	<i>Zea mays</i> , U06831
SEQ -ID-NO-47	Pulp Down regulated	U-D50	1.3	Basic Chitinase	60.2	678	Cowpea ( <i>Vigna unguiculata</i> ), X88801
SEQ -ID-NO-48	Pulp Down regulated	U-D86	1.7	Basic Chitinase	63	578	Cowpea ( <i>Vigna unguiculata</i> ), X88801
SEQ -ID-NO-49	Pulp Down regulated	U-D90	1.3	Basic Chitinase	61.8	670	Cowpea ( <i>Vigna unguiculata</i> ), X88801
SEQ -ID-NO-50	Pulp Down regulated	U-D93	0.7	Basic Chitinase	61.6	701	Cowpea ( <i>Vigna unguiculata</i> ), X88801
SEQ -ID-NO-51	Pulp Down regulated	U-D61	1	Beta-glucanase	61.9	795	Barley ( <i>Hordeum vulgare</i> ), X52572
SEQ -ID-NO-52	Pulp Down regulated	U-D63	2.3	Nodulin Gene	50.4	637	Lucerne ( <i>Medicago sativa</i> ), X13287

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Sequence Identity	Group	Clone no.	Size Kb	Published Sequence gene Identity	% Identity	Sequence Bp	Published Sequences
SEQ-ID-NO-53	Pulp Down regulated	U-D65	0.9	Extensin	56.7	556	Cowpea ( <i>Vigna unguiculata</i> ), X91836
SEQ-ID-NO-54	Pulp Down regulated	U-D84	0.8	Extensin	58.3	492	Cowpea ( <i>Vigna unguiculata</i> ), X91836
SEQ-ID-NO-55	Pulp Down regulated	U-D75	1.2	Wali 7	74.4	703	Wheat ( <i>Triticum aestivum</i> ), L28008
SEQ-ID-NO-56	Pulp Down regulated	U-D83	1.2	Wali 7	72.8	743	Wheat ( <i>Triticum aestivum</i> ), L28008
SEQ-ID-NO-57	Pulp Down regulated	U-D64	2.3	-	-	-	-